Check off steps as you complete them and don't be afraid to ask questions!

All centrifugation steps should be carried out at 16,000 x g (around 13K RPM in a typical microcentrifuge). This ensures all traces of the buffer are eluted at each step.

- [ ] **Excise the DNA fragment to be purified from the agarose gel** using a razor blade, scalpel or other clean cutting tool. Use care to trim excess agarose. Transfer it to a 1.5 ml microcentrifuge tube and weigh the gel slice.

    Note: Using UV light to visualize the slice is common, but exposure time should be kept as short as possible to minimize damage to the DNA. Use long-wave UV when possible, as shorter wavelengths induce greater damage. Also, trim off excess agarose from the perimeter of the band to minimize the amount of dissolving buffer needed, and to reduce the time necessary to extract the DNA.

- [ ] Add **4 volumes of Monarch Gel Dissolving Buffe**r to the tube with the gel slice (e.g., 400 µl buffer per 100 mg agarose). If the gel slice is >150 mg, consider reducing the amount of Gel Dissolving Buffer to 3 or 3.5 volumes to minimize the guanidine salt present in the workflow.

    Note: If the volume of the dissolved sample exceeds 800 µl, the loading of the sample onto the column should be performed in multiple rounds to not exceed the volume constraints of the spin column.

- [ ] **Incubate the sample between 37–55°C** (typically 50°C), inverting periodically until the gel slice is completely dissolved (generally 5–10 minutes).

    Note: For DNA fragments > 8 kb, an additional 1.5 volumes of water should be added after the slice is dissolved to mitigate the tighter binding of larger pieces of DNA (e.g., 100 mg gel slice: 400 µl Gel Dissolving Buffer: 150 µl water). Failure to dissolve all the agarose will decrease the recovery yield due to incomplete extraction of the DNA and potential clogging of the column by particles of agarose.

- [ ] Insert the column into the collection tube and **load the sample onto the column. Spin for 1 minute**, then discard flow-through.

- [ ] Re-insert column into collection tube. **Add 200 µl DNA Wash Buffer and spin for 1 minute.** Discarding flow-through is optional.

- [ ] Repeat the previous step.

- [ ] **Transfer column to a clean 1.5 ml microfuge tube**. Use care to ensure that the tip of the column has not come into contact with the flow-through. If in doubt, re-spin for 1 minute before placing into a clean microfuge tube.

- [ ] Add **≥ 6 µl of DNA Elution Buffer** to the center of the matrix. Wait for 1 minute, and spin for 1 minute to elute DNA.

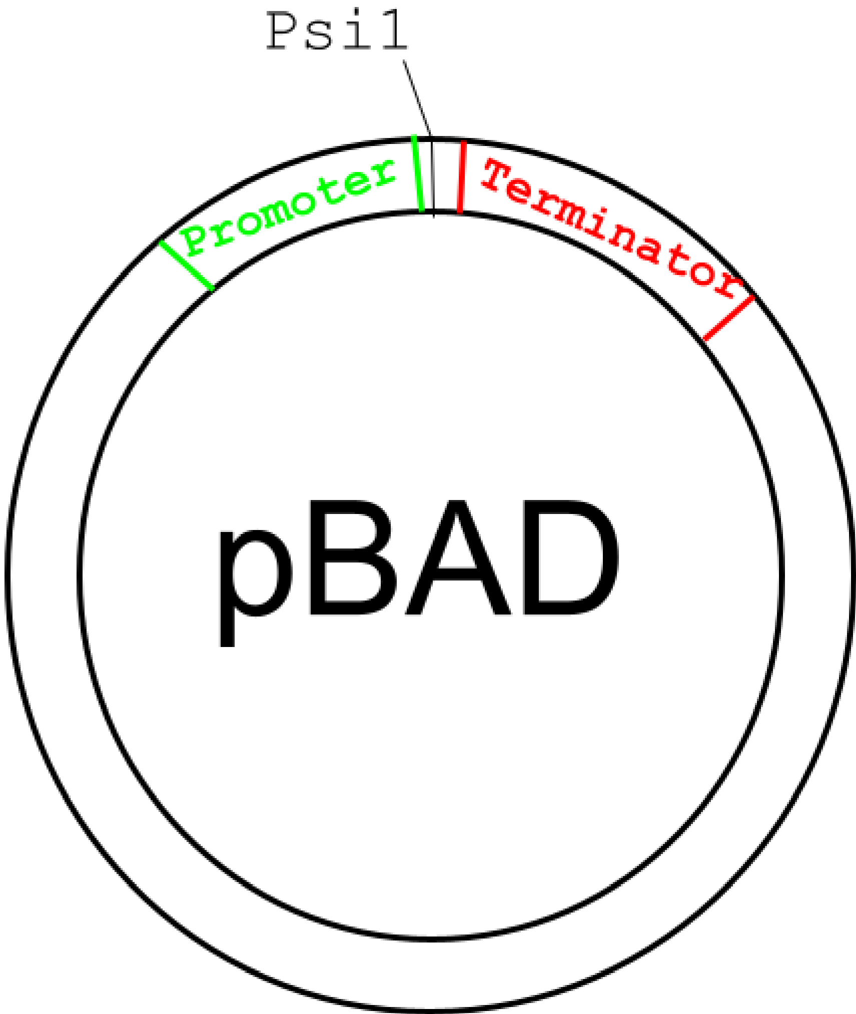
Note: Typical elution volumes are 6–20 µl. Nuclease-free water (pH 7–8.5) can also be used to elute the DNA. Yield may slightly increase if a larger volume of DNA Elution Buffer is used, but the DNA will be less concentrated. For larger size DNA (≥ 10 kb), heating the elution buffer to 50°C prior to use can improve yield. Care should be used to ensure the elution buffer is delivered onto the matrix and not the wall of the column to maximize elution efficiency.

Help out your labmates on the following problems they have encountered:

1. Jim nanodroped his gel purification and got a concentration of DNA that is too low for his next experiment. He reports cutting a bright band, and eluting with 20ul of elution buffer. What would you advise him to do instead next time?

2. Sally has gel purified a DNA fragment of ~14kb but got a very low yield. She reports dissolving a 100 mg gel slice with 400 µl Gel Dissolving Buffer at 37 for 5 minutes before loading the sample into the collection tube. She also reports using 6ul of room temperature elution buffer. What would you advise her to do instead next time?

<u>Bonus question:</u>
Your labmate Alex says they know a secret to getting high yield gel purifications. The secret is to use a spin column from a miniprep kit instead of the spin column in the gel purification kit. Is this valid?


Psi1
Promoter
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